"The Relations between the Hybrid and Parent Forms of Echinoid Larvæ." By H. M. VERNON, M.A., M.B. Communicated by Professor RAY LANKESTER, F.R.S. Received March 29,—Read May 5, 1898.

(From the Zoological Station, Naples.)

(Abstract.)

The object of this research was to determine systematically during a period of several months' duration, the exact relationship of structure and size existing between certain hybrid and parent Echinoid larval forms. Eight different species of Echinoids were worked with, but the larger number of observations were confined to three of them, viz., *Strongylocentrotus lividus*, *Sphærechinus granularis*, and *Echinus microtuberculatus*. The method of procedure was similar to that described in a former paper.* It consisted in shaking pieces of the ovaries and testes of the various Echinoids in small jars of water, and then mixing portions of the contents. In the cross-fertilisations, precautions were of course taken to prevent any accidental direct fertilisation of the ova. After standing an hour, the now fertilised ova were transferred to large jars of water, holding, as a rule, 2 to $3\frac{1}{2}$ litres. Here they were allowed to develop for eight days, when the plutei formed were killed, preserved, and examined under the microscope. The structure of the hybrids, in relation to that of the parent forms, was studied; and all the larvæ, both pure and hybrid, were measured by means of a micrometer eye-piece in respect of their body length and anal arm length. Fifty larvæ were, as a rule, measured in each case. In addition, the ova were examined under the microscope twenty-four hours after fertilisation, and the numbers of blastulæ and unfertilised ova in a given volume of water counted. Again, after eight days, the number of plutei surviving was similarly estimated.

Upon the cross *Sphærechinus* ♀ *Strongylocentrotus* ♂, twenty-two experiments were made. As a rule only about 10 per cent. of the ova were fertilised, and only 1 per cent. of them reached the eight days pluteus stage. The hybrids were most easily obtained in the summer months, few or none of the ova being cross-fertilised in the winter, unless the aids to fertilisation made use of by O. and R. Hertwig,† and by Born,‡ were adopted. Thus the former observers showed that if the ova were shaken in water and kept some hours, so that their vitality became diminished, they underwent cross-fertilisation

* 'Phil. Trans.,' B (1895), p. 577.

† 'Jenaische Zeitschrift f. Medicin,' vol. 19, p. 121 (1886).

‡ 'Pflüger's Archiv,' vol. 32, p. 453.

much more readily than when freshly shed. Born, on the other hand, found the cross fertilisation could be increased by increasing the amount of sperm added. Both methods, but especially the former, were found of value in the present research.

As regards the structure of the hybrids under discussion, it was found that the majority of those obtained in May, June, and July were of an almost pure *Sphærechinus* type, only a third or less of them being of an intermediate or *Strongylocentrotus* type. In November, on the other hand, only about a sixth were of the maternal, and five-sixths of the paternal type. Finally, in December and January, all the hybrid larvæ were of the paternal type. These latter larvæ in almost all cases showed obvious traces of their hybrid origin, but they were evidently much more inclined to the *Strongylocentrotus* than to the *Sphærechinus* type. Thus the body skeleton was like that of *Strongylocentrotus*, only it generally had a few abnormal projections springing from it. It was also, as a rule, about 25 per cent. shorter. The anal arm skeleton generally consisted of two rods, but there were very seldom any cross bars joining them, such as occur in *Sphærechinus* larvæ.

On the reciprocal cross of *Strongylocentrotus* ♀ and *Sphærechinus* ♂ eighteen experiments were made. During April, May, and June a fair number of the ova were cross-fertilised, but no plutei were obtained. In July and August some 47 per cent. of the ova were fertilised, and 29 per cent. of them survived to the eight days pluteus stage. In November and December, on the other hand, with one exception, not only were no plutei obtained, but as a rule not a single ovum was cross-fertilised. The hybrid larvæ themselves were of the pure *Strongylocentrotus* type, but in one instance the arms of the larvæ were very much longer than had ever been noticed in any other case. These hybrids appeared therefore to be of the nature of sport.

These extraordinary variations in the capacity for cross-fertilisation seem to be due to the variations in maturity which the sexual products undergo with change of season. Thus in the summer months most of the *Strongylocentrotus* individuals contain but very small quantities of ripe sexual products, or none at all. Also from the fifty different series of observations made on normal *Strongylocentrotus* larvæ, it appeared that the size of the larvæ kept at the maximum from the beginning of April till the beginning of May, but then began to dwindle down, so that at the beginning of July it reached its lowest level. The larvæ were now sometimes 30 per cent. smaller than the spring larvæ. After the middle of August they gradually increased again, and by the end of November had attained their maximum size. *Sphærechinus* larvæ, on the other hand, kept at about the same size throughout the year, though considerably

smaller numbers of the ova reached the pluteus stage in the summer than in the winter months. We see therefore that the Strongylocentrotus ♀-Sphærechinus ♂ *hybrid is only formed at the time when the* Strongylocentrotus *ova have reached their minimum of maturity; whilst in the case of the reciprocal hybrid, it follows that as the maturity of the* Strongylocentrotus *sperm increases, it is able to transmute first a portion and then the whole of the hybrid larvæ from the* Sphærechinus *to its own type. In other words, the characteristics of the hybrid offspring depend directly on the relative degrees of maturity of the sexual products.*

As a result of the ten experiments made on the cross *Echinus* ♀-*Strongylocentrotus* ♂, it was found that the hybrid larvæ were on an average about 8 per cent. *larger* than the pure parental larval forms, and, moreover, that even more of the cross-fertilised ova developed to plutei than of the directly fertilised ones. The hybrid larvæ were of a variable, but more or less intermediate, type. In the reciprocal cross, on the other hand, only a small proportion of the ova underwent fertilisation, and only about 1 per cent. of them reached the pluteus stage. These plutei were on an average 13·2 per cent. smaller than the pure maternal larvæ. In structure they were of a *Strongylocentrotus*-intermediate type.

Hybrids between *Sphærechinus* ♀ and *Echinus* ♂ were obtained on only two occasions. The larvæ were of a very variable *Echinus*-intermediate type, and were much dwarfed. In the reciprocal cross, hybrids were also obtained only twice, but then about 60 per cent. of the ova reached the pluteus stage. These hybrids were of the pure *Echinus* type. On most other occasions a small number of the ova were cross-fertilised, but failed to develop to plutei.

Hybrid larvæ, of the maternal type, were obtained on crossing *Arbacia* ova with *Strongylocentrotus* and with *Echinus* sperm, but with *Sphærechinus* sperm only gastrulæ resulted. Hybrids were also obtained between *Arbacia* ♂ and *Strongylocentrotus*, *Sphærechinus*, and *Echinus* ♀. These hybrids were of the maternal types, but in some of the *Sphærechinus* hybrid larvæ the anal arm skeletons were similar to those in pure *Arbacia* larvæ.

Hybrid larvæ were obtained between *Echinocardium cordatum* ♀ and *Strongylocentrotus*, *Sphærechinus*, *Echinus*, and *Arbacia* ♂, the larvæ being of the maternal type, but somewhat modified by the nature of the sperm. The hybrids between *Echinocardium mediterraneum* ♀ and *Strongylocentrotus* and *Echinus* ♂ were of an intermediate type; hence, one is afforded a physiological argument in favour of the specific difference of these two forms, the existence of which has hitherto been considered rather doubtful.

Hybrid larvæ of the maternal type were obtained on crossing *Strongylocentrotus* ♀ with *Dorocidaris* ♂, but the reciprocal cross

yielded only gastrulæ. Finally, hybrids, of a presumably intermediate character, were obtained from the cross *Echinus microtuberculatus* ♀ and *Echinus acutus* ♂. With the ova of *Sphærechinus* and *Echinocardium*, and the sperm of *Echinus acutus*, only gastrulæ were obtained.

On performing cross-fertilisations with the colour varieties of *Sphærechinus*, there was found to be a distinct diminution of fertility. In the most marked instance, obtained in the experiments made on June 2, it was found that when white-spined varieties were fertilised with white-spined, and violet-spined with violet, 98·5 per cent. of the ova reached the blastula stage, and 73 per cent. the eight days pluteus stage. But on cross-fertilising white-spined with violet-spined individuals, only 68 per cent. of the ova developed to blastulæ, and 15·6 per cent. to plutei. Also these crossed larvæ were 4·5 per cent. smaller than the uncrossed. Other series of experiments were made in July, November, and December, the differential fertility seeming to gradually diminish with the progress of the season. Nevertheless, it was always most distinctly present. On crossing the less definitely marked colour varieties of *Strongylocentrotus*, a small amount of infertility seemed to be present in one series of experiments, but none at all in another.

May 12, 1898.

The LORD LISTER, F.R.C.S., D.C.L., President, in the Chair.

Professor Dewar made a preliminary communication "On the Liquefaction of Hydrogen and Helium."

He prefaced his statement by referring to a letter which he had addressed to the President on the 10th May, announcing to him the fact that he had succeeded in liquefying hydrogen in quantity, and that by means of the liquid hydrogen he had also liquefied helium.

The following Papers were also read:—

I. "On the Magnetic Susceptibility of Liquid Oxygen." By Professor FLEMING, F.R.S., and Professor JAMES DEWAR, F.R.S.

II. "A Study of the Phyto-Plankton of the Atlantic." By G. MURRAY, F.R.S., and V. H. BLACKMAN.

III. "The Electric Response of Nerve to a single Stimulus investigated with the Capillary Electrometer. Preliminary Communication." By Professor GOTCH, F.R.S., and G. J. BURCH.